An inducible transgene expression system for regulated phenotypic modification of human embryonic stem cells.

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Public Summary:

Scientific Abstract:
Self-renewing pluripotent human embryonic stem (hES) cells are capable of regenerating such non-dividing cells as neurons and cardiomyocytes for therapies and can serve as an excellent experimental model for studying early human development. Both the spatial and temporal relationships of gene expression play a crucial role in determining differentiation; to obtain a better understanding of hES cell differentiation, it will be necessary to establish an inducible system in hES cells that enables specific transgene(s) to reversibly and conditionally express (1) at specific levels and (2) at particular time points during development. Using lentivirus (LV)-mediated gene transfer and a tetracycline-controlled trans-repressor (TR), we first established in hES cells a doxycycline (DOX)-inducible expression system of green fluorescent protein (GFP) to probe its reversibility and kinetics. Upon the addition of DOX, the percentage of GFP(+) hES cells increased time dependently: The time at which 50% of all green cells appeared (T(50)(on)) was 119.5+/-3.2 h; upon DOX removal, GFP expression declined with a half-time (T(50)(off)) of 127.7+/-3.9 h and became completely silenced at day 8. Both the proportion and total mean fluorescence intensity (MFI) were dose-dependent (EC(50)=24.5+/-2.2 ng/ml). The same system when incorporated into murine (m) ES cells similarly exhibited reversible dose-dependent responses with a similar sensitivity (EC(50)=49.5+/-8.5 ng/ml), but the much faster kinetics (T(50)(on)=35.5+/-5.5 h, T(50)(off) = 71.5+/-2.4 hours). DOX-induced expression of the Kir2.1 channels in mES and hES cells led to robust expression of the inwardly rectifying potassium (K(+)) current and thereby hyperpolarized the resting membrane potential (RMP). We conclude that the LV-inducible system established presents a unique tool for probing differentiation.